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Genetic diversity, phylogeny and conservation of the Javan rhinoceros (*Rhinoceros sondaicus*)

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Abstract

With a total population of less than 60 individuals limited to two locations, the Javan rhinoceros is perhaps the most endangered large mammal on earth. Although species specific information is crucial to its conservation, its precarious status, habitat inaccessibility, and behavioral adaptations pose major obstacles to its study. Here we report on the first genetic analysis of the two extant populations, in Ujung Kulon, Indonesia, and Cat Tien, Vietnam, and discuss their conservation. As its critically endangered status precluded invasive sampling, we extracted DNA from dung, amplifying and sequencing segments of the mtDNA 12S rRNA gene and the non-coding D-loop. Divergence between Javan rhinos from Ujung Kulon and Cat Tien was similar to that between recognized subspecies of African rhinos, and exceeded that between Sumatran rhinos. The Ujung Kulon and Cat Tien populations represent separate Evolutionary Significant Units, advocating independent management. However, given the precariousness of the Cat Tien population, demographic considerations may override genetic issues in the short term. Genetic diversity of Javan rhinos was low and population expansion in the immediate future will be critical for its survival.

Introduction

The Javan rhinoceros, *Rhinoceros sondaicus*, arguably the rarest large mammal on earth, is listed as 'Critically Endangered', and has been on Appendix I of CITES since 1975 (IUCN 2003). The entire species, numbering less than 60 individuals, is limited to two locations, Ujung Kulon in western Java (Indonesia) and Cat Tien in southern Vietnam. Its historical range extended from Assam in India, through Bangladesh and Indochina to the islands of Java and Sumatra (Figure 1). The Javan rhino's preferred habitat is lowland forest (Groves 1967; Foose and van Strien 1997), perhaps the most extensively exploited environment by expanding human populations. While extensive land use change was the ultimate cause of the Javan rhino's decline, as in other rhinoceros species, hunting for sport and commerce, accelerated its rapid spiral towards extinction (Groves 1967; Foose and van Strien 1997).

In the past, the Javan rhino may have existed in high local concentrations (Groves 1967). It was sufficiently numerous in 18th century Java to be considered an agricultural pest, and for government endorsement of its hunting (Ramono et al. 1993). Accounts from the 18th and 19th centuries document the shooting of many hundreds by

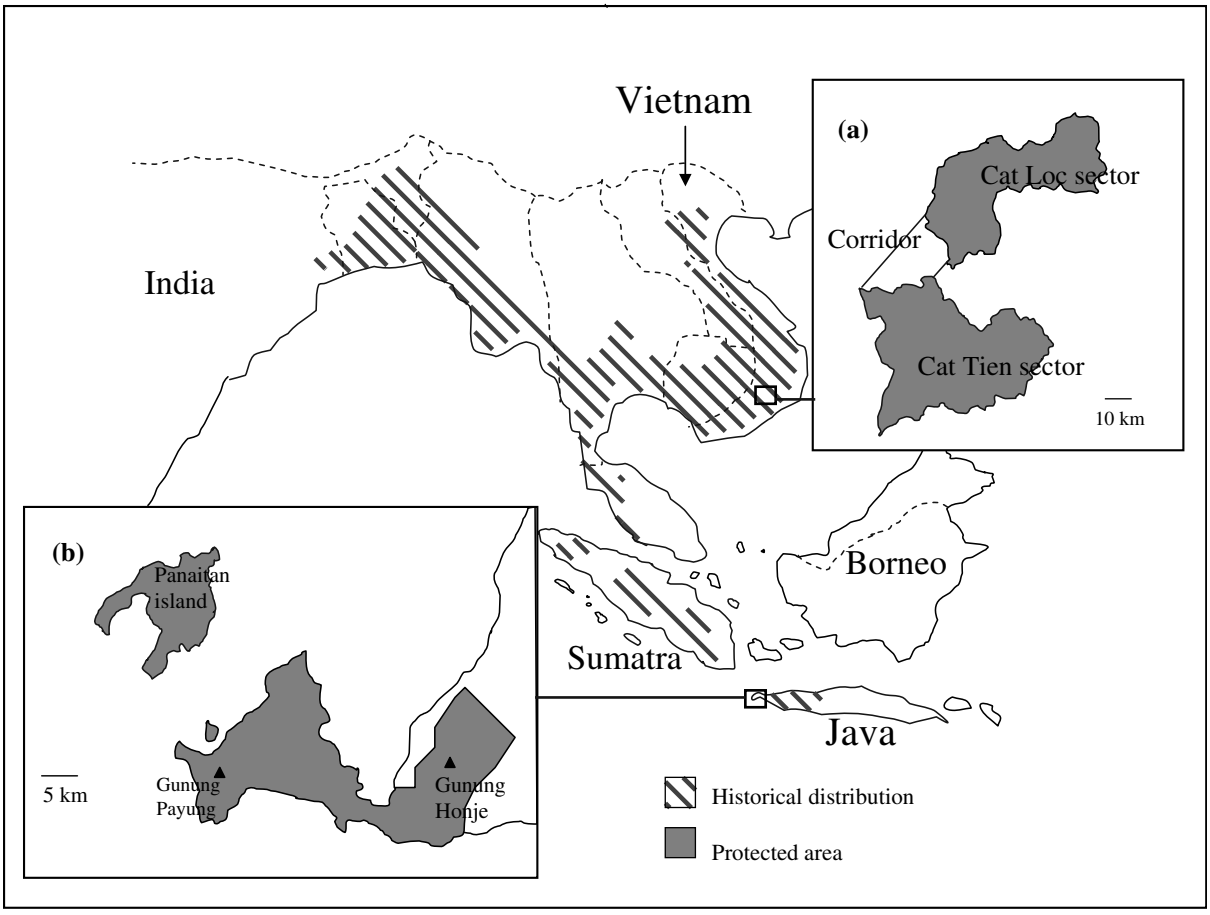

Figure 1. Historical distribution of the Javan rhinoceros. Insets show areas of current occurrence (a) Cat Tien National Park (b) Ujung Kulon National Park.

sportsmen (Groves 1967). By early 20th century, it had disappeared from almost its entire range (Groves 1967; Ramono et al. 1993). The last Javan rhino in Indonesia outside of Ujung Kulon, was shot in Karangnunggal in western Java in 1934 (Sadjudin 1992). Rumors of its survival on the mainland were validated by its 'rediscovery' in Vietnam (Santiapillai et al. 1993; Polet et al. 1999). However, subsequent surveys in the remotest reaches of its historical range have failed to locate any other surviving populations.

The approximately 300 km^2 Ujung Kulon peninsula is connected to Java by a narrow isthmus (Figure 1). Ujung Kulon was not resettled after the 1883 eruption of Krakatoa wiped out human settlements (Santiapillai and Suprahman 1986). An area including the peninsula was declared a National Park in 1980 (Ramono et al. 1993) (Figure 1). Poaching occurred in Ujung Kulon till the late 1960s, and a low of 25 surviving rhinos were estimated in 1967 (Ramono et al. 1993). Successful anti-poaching efforts saw the population increase to around 50 by 1980 (Ramono et al. 1993). Current camera trapping surveys estimate a population of around 40 (unpublished WWF technical report 2004). Rhinos are currently limited to the lowland areas in the peninsula. Although the peninsula is thought to have supported approximately 100 rhinos in the past, its carrying capacity may have decreased due to habitat change (Foose and van Strien 1995).

Recently poached Javan rhino parts found in a market place in 1988, indicated its survival in Cat Tien, southern Vietnam (Santiapillai et al. 1993; Polet et al. 1999) (Figure 1). Expeditions undertaken to verify its existence found fresh tracks and

dung, and recorded recent sightings by local people in Cat Loc area (Schaller et al. 1990; Santiapillai et al. 1993). Indirect evidence suggested the occurrence of approximately 10–15 rhinos at the time (Polet et al. 1999). Cat Loc was declared a rhinoceros refuge in 1992, but concurrent government policy changes in human resettlement resulted in a large influx of farmers around the same time (Raloff 1999). Cat Loc was integrated with the Cat Tien National Park in 1988 (Polet et al. 1999). Due to increased human pressure, rhino habitat decreased to roughly 15% of that available in 1990, and rhino numbers to an estimated 5–8 by 1999 (Raloff 1999; Polet et al. 1999). Latest estimates suggest that less than five rhinos are left (G. Polet pers. obs.; N. van Strien pers. com.). Currently many people live inside the protected area, and access to essential resources for rhinos remains unsecured.

The Javan rhinoceros ranks among the least known large mammals. Knowledge of its ecology and behavior is scant, with most information dating back to 18th and 19th century anecdotal accounts. While past records suggest it was a gregarious species (Groves 1967), currently they appear to be solitary (Polet et al. 1999; P. Fernando pers. obs.), perhaps a reflection of the extreme historical persecution and current low numbers. Javan rhinos have survived in Ujung Kulon and Cat Tien mainly due to habitat inaccessibility to humans (Ramono et al. 1993). In addition, surviving rhinos have become extremely cryptic and adept at avoiding people (Sadjudin 1992). These factors make direct study of surviving rhinos practically impossible. Currently there are no Javan rhinos in captivity. The Asian Rhino Specialist Group has strongly recommended the establishment of additional populations, and managed breeding centers located in natural habitat (Foose and van Strien 1997).

A study of 46 skulls examined by Groves (1967), suggested the occurrence of three Javan rhino subspecies, *R. s. inermis* in Sunderbans (Bangladesh), *R. s. floweri* in Sumatra and *R. s. sondaicus* in Java. Vietnam Javan rhinos were described as a fourth sub-species *R. s. annamiticus*, by Heude in 1892 (Groves and Guerin 1980). While evidence from skull morphometry is lacking (C. Groves pers. com.), based on footprint diameter, their body size is estimated to be 60–70% of Ujung Kulon animals (Polet et al. 1999).

The critically endangered status of the Javan rhino and logistic constraints, preclude invasive sampling. Previous genetic analysis of the species has been limited to a phylogenetic study that included a mtDNA sequence from a museum specimen (Tougard et al. 2001). Through PCR amplification of DNA from shed digestive tract cells passed out in dung (Kohn and Wayne 1997; Fernando et al. 2000, 2003), we sequenced segments of the 12S rRNA and the non-coding D-loop of Javan rhino mtDNA. Our objectives were to evaluate (1) genetic divergence between the two extant populations, and (2) the extent of genetic variation present in each population and the species, based on mtDNA sequence data. We discuss here our findings with respect to conservation of the species.

Materials and methods

Samples

One hundred samples of Javan rhinoceros dung were obtained from Ujung Kulon National Park and 30 samples from Cat Tien National Park. Samples were collected opportunistically by park guards and WWF scientists, from across the entire area from which rhinos are known. Approximately 5–10 g of dung was collected into a 15 ml vial and 95% ethanol added to cover the sample. Samples were stored and shipped at ambient temperature.

Three samples of horn and one of skin were obtained from Vietnam. The horn samples were from three rhino horns said to be over 40 years old. The skin sample was from confiscated parts of a Javan rhino poached in 1988. A single sample of skin was obtained from a Javan rhino specimen in Bogor museum, Indonesia. While no specific collection data were available, it was probably collected in early 20th century from West Java.

A single sequence for a 12S mtDNA segment of the Javan rhino was obtained from GenBank (Accession No. AJ245724). The sample from which this sequence was obtained by Tougard et al. (2001), was a piece of rib from a skeleton in the Museum of Natural History France. The skeleton was a gift/exchange to the French museum from Temminck, former Director of the Leiden Museum in the Netherlands (D. Robineau pers. com.). While no collection data was available, it is likely

to have been collected in the early 19th century from West Java (C. Smeenk and R. Pethiyagoda pers. com.). Samples for the other rhinoceros species consisted of tissue, details of which are provided in Table 1.

DNA extraction, PCR amplification and sequencing

DNA extraction from dung and tissue samples followed a protocol using proteinase K digestion, phenol/chloroform/isoamyl alcohol extraction, and QIAGEN column purification (Fernando et al. 2000, 2003).

PCR amplification was performed in 25μl reactions using 1μl DNA extract, 2μl of 100 mg/ml BSA, 2.5μl 10 $\times$ PCR buffer, 2.5μl 8 mM dNTP mix (Promega), 0.5μl 10μM primers, 0.1μl *Taq* DNA polymerase (Perkin Elmer Cetus) and 15.9μl water. Reactions were preceded by a 4 min denaturation step at 95 °C followed by 25 (tissue) or 45 (dung) cycles of, 1 min each at 55 °C for 12S, and 68 °C for D-loop; 72 °C extension; and 94 °C denaturation; followed by a 5 min 72 °C extension step, in a Perkin Elmer Cetus DNA Thermocycler. Primers were constructed based on the mitochondrial sequences of the Indian rhino (Accession No. X97336) and white rhino (Accession No. Y07726) obtained from GenBank. External primers for the 12S segment, RH-12S-F (GCC YAG ATG AGM CYA CCA RCT) and RH-12S-R (TAC RCT TAC CTT GTT ACG ACT) were situated in the tRNA-Phe and 12S rRNA genes respectively. Samples failing to give strong PCR products with the external primer pair (as visualized by electrophoresis of PCR products on ethidium bromide stained agarose gels), were amplified in four overlapping shorter fragments, using combinations of the external primers, and internal primers 12SA, 12SB, and 12SE, designed as universal primers for amphibia (Titus and Larson 1996). External primers for the D-loop segment RH-D-F1 (5′-CAT CAA CAC CCA AAG CTG AAA-3′) and RH-D-R1 (5′-ATG GGC CCG GAG CGA GAA CGA-3′) were located in tRNA-Pro and D-loop respectively.

Extractions from all 130 Javan rhinoceros dung samples were screened for D-loop amplification by PCR, and electrophoresis on agarose gels. Of the

Table 1. Taxa, origin, and material of the samples used in this study

Taxon	Common name	Country of origin	Locality of origin	Sample ID	No. of samples	Material
Rhinoceros sondaicus annamiticus	Javan rhinoceros (Vietnam)	Vietnam	Cat Tien National Park	None (wild)	30	Dung
		Vietnam	Unknown (confiscated)	None	3	Horn
		Vietnam	Unknown (confiscated)	None	1	Skin
Rhinoceros sondaicus sondaicus	Javan rhinoceros (Java)	Indonesia	Ujung Kulon National Park	None (wild)	100	Dung
		Indonesia	West Java?	Bogor Museum[b]	1	Skin
Rhinoceros unicornis	Indian rhinoceros	India/Nepal	None (captive born)[a]	Studbook # 87, 238	2	Blood
Dicerorhinus sumatrensis sumatrensis	Sumatran rhino (Sumatra)	Indonesia	Riau and Bengkulu (Sumatra)	Studbook # 18, 33	2	Hair
Dicerorhinus sumatrensis harrisoni	Sumatran rhino (Borneo)	Malaysia	Sabah (Borneo)	Studbook # 17, 26	2	Hair
Ceratotherium simum simum	Southern white rhinoceros	South Africa	Kruger National Park	None (wild)	2	Tissue
Ceratotherium simum cottoni	Northern white rhinoceros	Zaire	Garamba National Park	San Diego Zoo NX# 28818	1	Tissue
Diceros bicornis michaeli	Kenyan black rhinoceros	Kenya	Solio Game Reserve	None (wild)	2	Blood
Diceros bicornis minor	Southern black rhinoceros	Zimbabwe	Zambezi Valley	None (wild)	2	Blood

[a]Indian rhinos are restricted to India and Nepal.
[b]Sample ID details not available.

samples that amplified best, 10 (five each from Ujung Kulon and Cat Tien) were selected for amplification of the 12S segment. Amplification of both mtDNA segments (12S and D-loop) was attempted from the tissue samples.

Amplification products were sequenced with external and internal primers in both forward and reverse directions. Sequences were run on an ABI 377 automated sequencer using dye terminator cycle sequencing (Applied Biosystems).

Data analysis

Sequences were assembled and edited using the program Sequencher 4.1 (Gene Codes Corporation). The 12S fragments were aligned based on the secondary structure of mammalian 12S rRNA (Springer and Douzery 1996), with gaps preferentially inserted within loops to achieve alignment. Phylogenetic trees were constructed by using maximum parsimony and neighbor-joining procedures with PAUP*4.0b10 (Swofford 1998). Maximum parsimony analysis was conducted using a heuristic search with equal weighting, gaps treated as a fifth state, random stepwise addition of taxa (10 replicates), and TBR branch swapping. Neighbor-joining analysis was conducted using uncorrected 'p' distances. The following sequences obtained from GenBank were used as outgroups for the 12S analysis: donkey (*Equus asinus*) NC 001788, horse (*Equus caballus*) X79547, Indo-Malayan tapir (*Tapirus indicus*) AY012148; and the mountain tapir (*Tapirus pinchaque*) AF038012. Because of excessive alignment ambiguity from the high level of divergence in the D-loop, the Indian rhino alone was used as an out-group in analyzing Javan rhino D-loop sequences. The robustness of phylogenetic hypotheses was tested as percentage recurrence of clusters based on 1000 bootstrapped replications with PAUP*.

Results

12S rRNA

A 937 bp mtDNA fragment was amplified from tissue samples of black, white, Indian and Sumatran rhinos. The same 12S fragment was successfully amplified in shorter lengths and assembled, from all 10 Javan rhino dung samples (five each from Ujung Kulon and Cat Tien), and the three Javan rhino tissue samples that provided D-loop sequence. Due to sequence ambiguity at the ends of some amplified fragments, only 840 bases of the 12S rRNA gene were used in the analysis. The first base of the analyzed fragment corresponded to base No. 103 of the *C. simum* reference sequence (GenBank Accession No. Y07726). 12S rRNA sequences were deposited in GenBank (Accession Nos. AY739616–AY739624).

All five dung samples from Ujung Kulon and the Bogor Museum sample provided a single haplotype, identical to the published Javan rhino sequence (Tougard et al. 2001). All five dung samples from Cat Tien, and the horn and skin samples from Vietnam produced a single haplotype that differed from the Ujung Kulon haplotype by four transitions. Sequence divergence in the 12S segment between the two Javan rhinoceros haplotypes from Ujung Kulon and Cat Tien was 0.5% (Table 2). Divergence between the subspecies of white rhinoceros (*C. s. simum* and *C. s. cottoni*) was 0.9% and that between the black rhinoceros (*D. b. michaeli* and *D. b. minor*) 0.5%. No sequence divergence was observed in the analyzed segment between the Sumatran rhinoceros subspecies *D. s. sumatranus* and *D. s. harrissoni*. Interspecies divergence in the analyzed 12S segment, between Javan and Indian rhinoceros was 2.4–2.7% (Table 2). Both the neighbor-joining and maximum parsimony analyses produced trees with similar topology, clustering Javan and Indian rhinos as members of a one-horned rhinoceros clade (Figure 2).

D-loop

The D-loop primers amplified a 413 bp fragment comprising 21 bp from the 3′ end of tRNA-Pro, and 392 bp of the adjacent D-loop. Of dung samples collected from Ujung Kulon and Cat Tien, a total of 39% ($n = 39$) and 47% ($n = 14$), respectively, provided sequence. Amplification and sequencing was successful from the tissue samples of black, white, Indian and Sumatran rhinos, and three Javan rhino tissue samples (Bogor Museum sample, one horn sample and the skin sample from Vietnam). No amplification products were obtained from two Vietnam Javan rhino horn samples. D-loop sequences were deposited in

Table 2. Uncorrected p distance matrices derived from 12S rRNA (below diagonal) and D-loop (above diagonal) data sets

	Rsa	*Rss* I	Rss*II*	*Rss* III	*Ru*	*Csc*	*Css*	*Dbmc* I	*Dbmc*II	*Dbm* I	Dbmm*II*	*Dsh*	*Dss*
Rhinoceros sondaicus annamiticus[a]	–	0.051	0.048	0.053	0.120	0.197	0.187	0.196	0.203	0.183	0.181	0.193	0.193
Rhinoceros sondaicus sondaicus I	0.005	–	0.002	0.002	0.137	0.201	0.187	0.201	0.208	0.194	0.186	0.208	0.208
Rhinoceros sondaicus sondaicus II	0.005	0	–	0.005	0.135	0.204	0.184	0.203	0.211	0.196	0.189	0.208	0.208
Rhinoceros sondaicus sondaicus III	0.005	0	0	–	0.140	0.204	0.189	0.203	0.211	0.196	0.189	0.211	0.211
Rhinoceros unicornis[a]	0.024	0.027	0.027	0.027	–	0.216	0.211	0.228	0.236	0.206	0.208	0.203	0.203
Ceratotherium simum cottoni	0.051	0.053	0.053	0.053	0.053	–	0.072	0.155	0.155	0.169	0.167	0.245	0.245
Ceratotherium simum simum[a]	0.054	0.057	0.057	0.057	0.057	0.009	–	0.140	0.140	0.140	0.142	0.253	0.253
Diceros bicornis michaeli I	0.056	0.058	0.058	0.058	0.056	0.035	0.039	–	0.007	0.034	0.036	0.256	0.256
Diceros bicornis michaeli II	0.056	0.058	0.058	0.058	0.056	0.035	0.039	0	–	0.041	0.043	0.263	0.263
Diceros bicornis minor I	0.061	0.063	0.063	0.063	0.061	0.038	0.042	0.005	0.005	–	0.007	0.255	0.255
Diceros bicornis minor II	0.061	0.063	0.063	0.063	0.061	0.038	0.042	0.005	0.005	0	–	0.253	0.253
Dicerorhinus sumatrensis harrisoni[a]	0.057	0.062	0.062	0.062	0.058	0.059	0.061	0.062	0.062	0.064	0.064	–	0
Dicerorhinus sumatrensis sumatrensis[a]	0.057	0.062	0.062	0.062	0.058	0.059	0.061	0.062	0.062	0.064	0.064	0	–

[a] All individuals sequenced provided the identical sequence for both 12S and D loop fragments.

Figure 2. Maximum parsimony phylogram showing the phylogenetic relationships of the extant rhinoceros taxa based on 12S rRNA sequences. The numbers on branches represent bootstrap support.

GenBank (Accession Nos. AY739625–AY739628 and AY742825–AY742833).

Two haplotypes (I and II) differing from each other by a single transition were obtained from dung samples from Ujung Kulon. Haplotype I was found in 44% ($n=17$) and haplotype II in 57% ($n=22$) of the samples. The skin sample from the Bogor Museum yielded a third haplotype (III), differing by one and two transitions respectively from haplotypes I and II. In view of the close relationship between the Bogor Museum and Ujung Kulon D-loop haplotypes, and the identical 12S sequence obtained from the Bogor museum sample and Ujung Kulon samples, the Bogor museum D-loop haplotype was tentatively assigned to the same subspecies and designated *R. s. sondaicus* (Figure3).

A single haplotype was obtained from all 16 samples from Vietnam (dung and tissue), which differed from haplotypes I to III by one transversion and 19–20 transitions. Table 2 provides genetic distances between all rhinoceros taxa analyzed.

Alignment of the four Javan rhinoceros haplotypes was achieved without indels. Maximum parsimony analysis recovered a single most parsimonious tree identical to that from neighbor-joining analysis. Haplotypes I–III formed a

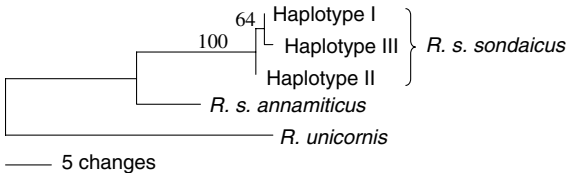

Figure 3. Maximum parsimony phylogram of Javan rhinoceros D-loop sequences. The numbers on branches represent bootstrap support. Haplotypes I and II are from Ujung Kulon and Haplotype III from a specimen in the Bogor museum.

monophyletic clade with *R. s. annamiticus* as the sister group (Figure 3). D-Loop sequence divergence between Ujung Kulon and Cat Tien populations of Javan rhinos was 4.8–5.1%. Sequence divergence between the subspecies of white rhinos (*C. s. simum* and *C. s. cottoni*) was 7.2%, black rhinos (*D. b. michaeli* and *D. b. minor*) 3.4–4.3%, and no divergence was observed between the Sumatran rhino (*D. s. sumatranus* and *D. s. harrissoni*) subspecies (Table 2).

Discussion

Biogeography

The interspecific divergence in *Rhinoceros* observed by us in the 12S analysis was 2.4–2.7%. Observed intra-specific divergence between Ujung Kulon and Vietnam *R. sondaicus* was 0.5%, or approximately one fifth the inter-specific divergence in *Rhinoceros*. The molecular divergence estimate based on the 12S gene by Tougard et al. for *R. sondaicus* and *R. unicornis*, was 11.7 ± 1.9 Myr, and the corresponding paleontological estimation quoted, 3.3–1.6 Myr (Tougard et al. 2001). Calibrating our observed inter-species divergence in *Rhinoceros* using both estimates, and assuming the same rate of divergence within *R. sondaicus*, Javan rhinos from Ujung Kulon and Cat Tien shared a common ancestor in the Plio-Pleistocene, around 2 Myr to 300,000 year ago.

In antiquity, Java was part of a single land mass called Sundaland that was continuous with the mainland (Holloway and Hall 1998). Sea level fluctuation during Pleistocene glaciations periodically disconnected and reconnected the Sunda islands with the mainland through submersion and emergence of low lying parts of the Sunda shelf (Holloway and Hall 1998), with attendant population isolation. Thus, the observed divergence between the Java (Ujung Kulon) and mainland (Cat Tien) Javan rhinos is consistent with regional geologic history.

Subspecific taxonomy

The observed genetic divergence between the Ujung Kulon and Cat Tien populations of Javan rhino, in both the 12S rRNA and D-loop segments is comparable to that between recognized subspecies of both white rhinos and black rhinos, and exceeds that between subspecies of Sumatran rhinos. Therefore, our results support the subspecific classification of the Ujung Kulon and Cat Tien Javan rhinos as *R. s. sondaicus* and *R. s. annamiticus*, respectively.

The possibility that the observed geographic genetic partitioning reflects stochastic elimination of co-existing divergent haplotypes through population decline cannot be ruled out. However, the extent of divergence between the Java and Vietnam populations, its concordance with the subspecific taxonomy and geologic history, and the congruence of present day Ujung Kulon haplotypes with that from museum specimens suggest otherwise.

Genetic variability

While Java Javan rhinos were monomorphic for the analyzed 12S rRNA segment analyzed, three haplotypes were observed in the D-loop segment. The non-coding D-loop evolves much faster than the protein coding 12S rRNA gene, hence greater diversity and divergence is expected in the D-loop. Two D-loop haplotypes were observed in Ujung Kulon at similar frequency, and a single haplotype in Cat Tien. Thus, the extent of mtDNA variability in the two Javan rhino populations was low but not unexpected given their size. If the Bogor Museum specimen did originate from Java, its unique D-loop haplotype indicates a somewhat greater historical genetic diversity within Java.

The catastrophic explosion of Krakatoa in 1883, and its aftermath, in addition to annihilating the human settlements in Ujung Kulon, would likely have exterminated the Javan rhinos then inhabiting the peninsula. The present day Javan rhino population probably originated from subsequent re-colonization. Therefore, it is likely to have been subject to founder effects, especially

since the species was already in decline. The Ujung Kulon population has been demographically 'closed' for at least the past 70 years, and subject to a bottleneck of about 25 individuals in 1967, and a persistent low population size of less than 50 individuals since. As effective population sizes are usually lower than corresponding census population sizes (Hartl and Clark 1989), the impact on genetic variability is even more drastic than apparent from the census data. Thus, Javan rhino genetic diversity in Ujung Kulon is likely to have suffered extreme genetic drift, from the additive effects of founder events, bottlenecks, and persistent small population size. Given such exceptional negative influences, it is indeed surprising that the population still harbors two haplotypes. In comparison, the single haplotype observed in Cat Tien from current and older samples indicates lower genetic diversity in Javan rhinos in Vietnam than in Java. However, given the extremely small number of individuals in Vietnam, the singular origin of all analyzed samples, and their comparatively contemporaneous nature, the lack of diversity is expected and may not reflect historical diversity.

Conservation and management: Ujung Kulon

The main concern for Javan rhino conservation in Ujung Kulon is the possible decrease in carrying capacity over the past few decades, leading to population stasis, or even gradual decline. While our results indicate the existence of some genetic diversity in Ujung Kulon, persistent small population size will inevitably lead to continued genetic erosion. Therefore, removing the limiting factors in its environment, and resuming population growth is essential for conservation of the species. While the occurrence of the entire Java Javan rhino population in a peninsula 300 km^2 allows for greater protection, and could reduce detrimental consequences of rarity such as Allee effects (Stephens and Sutherland 1999), it is also potentially dangerous, as environmental stochasticity or a disease outbreak could precipitate its extinction. Thus, both in terms of possible resource limitations in Ujung Kulon, and the proverbial 'all the eggs in a single basket' concept, establishing a second population of the Java Javan rhino is an extremely urgent need.

Conservation and management: Cat Tien

As the Cat Tien population is practically on the verge of extinction, translocating a few individuals from Ujung Kulon to rescue the population is an option worth considering. However, the apparent genetic divergence between the Ujung Kulon and Cat Tien populations creates a dilemma. Our findings suggest that the two populations represent separate Evolutionary Significant Units (ESU), and support their designation as two separate subspecies. Clearly the two populations have separate evolutionary trajectories, advocating independent management. Separate management was recommended for the two Sumatran rhino subspecies *D. s. sumatrensis* and *D. s. harrisoni* in view of their genetic divergence and possible detrimental effects of gene flow between long separated genetically distinct conspecifics (Morales et al. 1997). The Ujung Kulon and Cat Tien rhinos are much more divergent than the two Sumatran rhino subspecies. Therefore, the two Javan rhino populations should be managed separately, subject to the caveat that there is a realistic chance of survival of both populations on their own.

Recent surveys have suggested that the Cat Tien population is extremely close to extinction, perhaps numbering less than five individuals (G. Polet pers. obs.; N. van Strien pers. com.). Faced with the real possibility that demographic or environmental stochasticity will cause the extinction of the Vietnam Javan rhino in the short term, cross breeding with Javan animals may be the only avenue for saving some of the genetic information inherent in the Vietnam population. Since the genetic divergence between Java and Vietnam animals suggests a low probability of success in cross-breeding, its advisability depends on the expected outcomes of alternate options. While maintaining the status quo is one option, the continuous decline since its rediscovery suggests that the Vietnam Javan rhino will soon go extinct without any additional gain in knowledge or conservation, through its pursuance. Thus, immediate confirmation of the number of rhinos left in Vietnam, identification of the causes of decline, realistic evaluation of the risks, benefits, and practicality of all possible management options, and urgent action is essential.

Ex-situ conservation

The failure of the Sumatran rhino captive-breeding program sets an unfavorable precedent for attempting ex-situ conservation of the Javan rhino. Its critical conservation status makes the risks of capture, restraint, transport, and captive management required for traditional ex-situ conservation unacceptable. However, the reported historical gregariousness and high local densities of the Javan rhino may indicate some similarity of its social system to Indian and white rhinos, two gregarious species that have made remarkable comebacks from the brink of extinction. This is in contrast to the black and Sumatran rhinos, which are less social and less responsive to active management. The broader diet of the Javan rhino suggests its ecological placement as a generalist herbivore, and historical crop raiding imply its ability to adapt to a human modified environment. Therefore, ecological and behavioral attributes of Javan rhinos may make them more amenable to active management than Sumatran rhinos. Less invasive forms of ex-situ conservation, such as managed rhino breeding centers in natural habitat (Foose and van Strien 1997), exploiting the natural movement of rhinos from the current protected area and management under semi-captive conditions (F. Bagley and N. van Strien pers. com), supplemental feeding, and encouragement of range expansion maybe of value in its conservation.

Conclusion

While genetic factors are important determinants of species' persistence (Frankham 2005), demographic factors are also of fundamental importance in the survival of small populations (Lande 1988). Conservation and management of the Javan rhino requires understanding of its demography, ecology, behavior, genetics, and their interactions. While obtaining data on the Javan rhino is extremely challenging, given its precariousness, this may be the last opportunity to do so. For both Javan rhino populations, attempts at range expansion or securing safe access to critical resources, are at variance with the livelihood of multitudes of people. Thus, funding of a scale befitting a priority global issue, by providing a real incentive and the means for governments to take appropriate actions is critical. The international conservation community needs to play a prominent role in assisting local authorities and scientists in achieving these objectives.

Extinction of the Javan rhino would represent the loss of a significant and irreplaceable segment of the rhinoceratid radiation. Allowing a species such as a rhinoceros to go extinct in the 21st century would be tragic and unpardonable.

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